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Female psychopharmacology matters! Towards a sex specific psychopharmacology

Short title: **Towards a sex specific psychopharmacology**

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Abstract

There is increasing recognition that women have a higher prevalence of certain psychiatric illnesses, and a differential treatment response and course of illness compared to men. Additionally, clinicians deal with a number of disorders like premenstrual syndrome (PMS), premenstrual dysphoric disorder (PMDD) and postpartum depression, which affect women specifically and for which treatment and biological pathways are still unclear. In this article we highlight recent research which suggests that different biological mechanisms may underlie sex differences in responsiveness to stress. Sex differences are evident at the receptor level; where corticotropin releasing factor receptor (CRF₁) shows differential coupling to adaptor proteins in males and females. The neuropeptide oxytocin also shows sex-specific effects in a range of social behaviours. It may act as a biomarker in post-traumatic stress disorder (PTSD) where sex differences are evident. Studies in women using hormonal contraception show that some of these oxytocin mediated effects are likely influenced by sex hormones. In female rats rapid changes in circulating progesterone levels are associated with exaggerated behavioural responses to mild stress and blunted response to benzodiazepines that could be prevented by acute treatment with low dose fluoxetine. Perceived barriers in research on women have hindered progress. The development of a sex specific psychopharmacology as a basis for translating this type of research into clinical practice is vital to improve treatment outcomes for women.

Word count-223

Keywords: sex differences, sex hormones, gender, oestrogen, estrogen, progesterone, neurosteroids, allopregnanolone, oxytocin.

Introduction

The World Health Organization recognizes sex as a crucial factor determining the prevalence and severity of mental health problems (World Health Organization, 2000). Some disorders affect women exclusively: approximately 20% of women of reproductive age experience premenstrual syndrome (PMS) and 3-8% of women experience premenstrual dysphoric disorder (PMDD) (Halbreich et al., 2003, Halbreich et al., 2007). Women are twice as likely to suffer from depression and anxiety disorders and have higher rates of attempted suicide (Mergl et al., 2015) while men have a higher risk of substance use disorders, some neurodevelopmental conditions and death by suicide (Eaton et al., 2012; McCarthy and Wright, 2017); (Bekker and van Mens-Verhulst, 2007; McCarthy, 2016; W.H.O, 2015). Social, cultural and economic inequalities across sexes have been found to account for some of these disparities (World Health Organization, 2000; Kuehner, 2017). For example, women are more frequently the primary victims of domestic violence, which is a risk factor for depression (World Health Organization, 2000). The fact that a proportion of these differences can be explained by sociocultural factors has somehow concealed the importance of biological determinants (Li and Graham, 2017).

An extensive body of literature from animal and human studies has appeared in the last twenty-five years describing biochemical sex differences in response to stress (McEwen and Milner, 2017; Carey et al., 1995; Ferrini et al., 1997). Given that stress is implicated in numerous psychiatric diseases including affective disorders, post-traumatic stress disorder and substance abuse, sex differences in stress response systems (McEwen, 2017) may underlie the different prevalence by sex seen in these disorders (Luine et al., 2017). Two

important stress response systems in which sex differences have been reported are the corticotropin-releasing factor (CRF) system and the brain norepinephrine system arising from the locus coeruleus (LC). Additionally, differential effects between the sexes have been found in the effectiveness, side effect profile and pharmacokinetics of psychotropic drugs (Bigos et al., 2009; Marazziti et al., 2013). As an example, zolpidem, one of the most widely prescribed drugs for insomnia is known to have lower clearance in women and the FDA recommends different doses for men and women (Greenblatt et al., 2014; FDA, 2014). However, despite growing evidence suggesting differential pharmacological effects, the underlying mechanisms remain woefully under-researched. In this review, we aim to highlight some specific aspects of the current research in the literature in this area, focusing specifically on sex differences in stress response systems such as corticotropin-releasing factor and the brain norepinephrine systems, female neurophysiology and the contribution of sex hormones, allopregnanolone and oxytocin to sex differences in stress-responsiveness and psychiatric illness. The authors contributed to a symposium on this topic held at the British Association for Psychopharmacology Summer Meeting 2016.

Stress-responsiveness and corticotropin releasing factor signalling

Life stressors have been implicated in many of the major psychiatric disorders that are more prevalent in females compared to males, including depression and post-traumatic stress disorder (PTSD)(Choi et al., 2016; van der Meer et al., 2016; Olf et al., 2007). Individuals with these disorders often exhibit features of a dysfunctional hypothalamic-pituitary-adrenal (HPA) axis (Arnett et al., 2016). As a result, most investigations into sex differences in neuropsychiatric disease have focused on stress hormones or the HPA axis. Psychiatric

diseases that are more prevalent in females also share symptoms of hyperarousal, characterized by sleep disturbances, restlessness, and inability to concentrate.

The major brain norepinephrine (NE) system that arises from the pontine nucleus locus coeruleus (LC) is a stress responsive structure that regulates arousal and cognitive responses to stress through a network of widely distributed projections to the cortex (Aston-Jones and Cohen, 2005; Valentino and Van Bockstaele, 2008; Valentino et al., 2013a). This system is activated in parallel with the HPA axis by many of the same triggers and its stimulation is associated with increased arousal, and cognitive flexibility. This LC response to stress is mediated by corticotropin-releasing factor (CRF), the same neuropeptide released by the HPA axis to initiate the endocrine limb of the stress response. The combined neurohormone and neurotransmitter function of CRF may coordinate endocrine and cognitive responses to life-threatening challenges. Although LC-NE activation would be adaptive in response to an acute stressor, inappropriate or persistent activation of this system could produce the pathological hyperarousal that characterizes stress-related psychiatric disorders.

In unstressed rats, the basic neuronal characteristics of LC neurons, such as spontaneous discharge rate and sensory-evoked discharge, are comparable between males and females (Curtis et al., 2006). However, LC neurons of female rats are sensitized to CRF and this is manifested as a greater activation by certain stressors (Curtis et al., 2006). Receptor immunoprecipitation studies revealed a molecular basis for this in the differential coupling of the CRF receptor (CRF₁) to adaptor proteins in males and females (Bangasser et al., 2010). For example, in unstressed rats, we found that CRF₁ showed greater association with its GTP-binding protein Gs, in females compared to males, consistent with a greater CRF-elicited neuronal activation. Like other G-protein coupled receptors, CRF₁ internalizes following

agonist binding and is transported into multivesicular bodies, a step towards CRF₁ downregulation (Reyes et al., 2006; Reyes et al., 2008). CRF₁ internalization is initiated by its association with β -arrestin-2. This process protects neurons from excessive CRF. In an experiment with rats exposed to swim stress, both increased CRF₁ coupling to β -arrestin-2 and CRF₁ internalization were found in males compared to females (Bangasser et al., 2010). The greater coupling of CRF₁ to Gs and the impaired ability of β -arrestin-2 to associate with CRF₁ is predicted to render LC neurons of females more sensitive to stress and less able to adapt by receptor internalization. In the absence of stress, sex differences in CRF receptor signalling would have no consequences because there is no tonic influence of CRF. However, sex differences in LC activity will be apparent and magnified in conditions in which CRF release is excessive as has been proposed in stress-related psychiatric disorders such as depression and post-traumatic stress disorder. This condition can be modelled in mice that are genetically modified to overexpress CRF (CRF-OE mice). Both male and female CRF-OE mice show hyperinnervation of the LC by CRF axon terminals and there is no sex difference in this innervation (Bangasser et al., 2012). Based on this, LC neuronal discharge rates are predicted to be higher in both male and female CRF-OE mice compared to wildtype animals. Recordings from LC slices of male and female wildtype revealed no sex difference in LC discharge rates. Surprisingly, LC neuronal discharge rates of male CRF-OE mice were similar to wildtype mice indicating that they were protected from CRF overexpression (Bangasser et al., 2012). In contrast, LC discharge rates of female CRF-OE mice were nearly three times greater than all other groups. Electron microscopic analysis suggested that this was due to a lack of CRF₁ internalization in females (Bangasser et al., 2012). Whereas CRF₁ was predominantly localized to the cytoplasm in male CRF-OE mice, in female CRF-OE mice it appeared mainly in the plasma membrane leaving the cells vulnerable to excessive CRF in the synapse. This

combination of increased sensitivity due to enhanced CRF₁-Gs binding and decreased ability to internalize CRF₁ as a result of decreased CRF₁- β -arrestin-2 association could make females more vulnerable to stress-related disorders.

Because Gs and β -arrestin-2 engage different cellular signalling pathways, the sex bias in CRF₁ coupling predicts that stress will initiate sex-specific cellular reactions in males and females (Valentino et al., 2013b). These can translate to sex specific physiological and behavioural responses to stress and sex-specific stress-related pathology. These sex differences would be magnified under conditions of elevated CRF release, which are believed to occur in certain stress-related psychiatric disorders that show a female prevalence, including depression and PTSD (Banki et al., 1987; Bremner et al., 1997; Nemeroff et al., 1984). Supporting this, male and female CRF-OE mice have distinct cortical phosphoproteomic profiles that can be accounted for in part by sex biases in CRF₁ coupling to Gs and β -arrestin-2 (Bangasser et al., 2016). Distinctions between these profiles may reveal substrates that are the basis for sex differences in the prevalence of stress-related diseases.

In addition to differences at the postsynaptic level, sex differences in afferents that mediate the response of the LC-norepinephrine system to stress could account for sex biases in stress-related diseases. Some of the circuitry underlying LC activation by different stimuli has been delineated (Van Bockstaele et al., 2001). For example, LC activation by hypotensive stress and social stress is mediated by CRF amygdalar afferents, while LC activation by colonic distention is mediated in part by Barrington's nucleus (Curtis et al., 2002; Reyes et al., 2015; Rouzade-Dominguez et al., 2001). The possibility that there are sex differences in CRF expression or release from these afferents requires future investigation.

Impact of sex hormones and allopregnanolone in PMS and PMDD.

During the luteal phase of the menstrual cycle, many women develop adverse psychological and physical symptoms collectively known as premenstrual syndrome (PMS). The most commonly reported psychological symptoms included anger and irritability, mood swings, tearfulness, fatigue, lack of energy, and food cravings (Ryu and Kim, 2015). Premenstrual Dysphoric Disorder (PMDD) is commonly characterized as a severe type of PMS. The current DSM-5 diagnosis requires a perimenstrual pattern of physical, behavioural and/or mood related symptoms with special emphasis on the severity of psychological symptoms (American Psychiatric Association, 2013). Typically, symptoms peak during the late luteal phase and resolve shortly after the onset of menstruation (Halbreich et al, 2003). The cyclical change in secretion of progesterone during the menstrual cycle may be one of the factors that contribute to the development of these negative mood states in susceptible women. Following ovulation progesterone secretion increases markedly, remains elevated during the luteal phase, before returning to basal levels immediately prior to menstruation (Fig 1-A). However, there is considerable individual variation in hormone levels and women who developed symptoms of PMS displayed a significantly different luteal phase salivary progesterone profile compared to women who remained asymptomatic (Lovick et al., 2017). Whereas early luteal phase progesterone concentration was similar in both groups, in women who remained asymptomatic progesterone showed a gradual linear decline throughout the mid and late luteal phase right up to the onset of menstruation (Fig 1-B). In contrast, in women who developed symptoms of PMS, progesterone remained at a relatively high and stable level during the early and mid-luteal phase before undergoing a sharp decline during the three days prior to menstruation (Fig 1-C) (Lovick et al., 2017).

Progesterone, as well as its neuroactive steroid metabolite allopregnanolone, passes readily through the blood brain barrier and in the brain its concentration parallels that in the plasma (Pardridge et al., 1980). Progesterone, via the action of allopregnanolone, exerts dose-related anxiogenic and anxiolytic effects in women, characterised by an inverted U-shaped dose-response relationship (Andréen et al., 2009). Interestingly, in post-menopausal women in whom endogenous progesterone concentration remains at a stable low level, daily administration of progesterone at doses sufficient to achieve a physiological concentration was associated with a progressive worsening of negative mood (Andréen et al., 2006). This response mimics the progressive worsening in severity of symptoms that occurs in PMS sufferers who display a stable elevated level of progesterone during the early to mid-luteal phase (Lovick et al., 2017). The mechanism that underlies the effect of prolonged exposure to progesterone is not completely understood but may be linked to tolerance effects [see (Turkmen et al., 2011) for a review of this topic].

Paradoxically, in women who developed PMS, symptoms worsened even further during the sharp decline progesterone concentration in the 3 days prior to menstruation (Lovick et al., 2017). In animal models, withdrawal from progesterone following prolonged exposure precipitated anxiogenic effects (Devall et al., 2009) (Smith et al., 1998). However, such effects were seen only when the withdrawal was rapid and were absent if the steroid concentration was allowed to fall gradually (Doornbos et al., 2009), in line with the absence of PMS symptoms in women in whom progesterone underwent a slow linear decline during the whole luteal phase (Lovick et al., 2017). It is well established in rats that a rapid decline in progesterone, either following cessation of prolonged dosing, or during the natural decline that occurs during the late diestrus phase, can precipitate changes in GABA_A receptor

expression associated with an increase in behavioural measures of anxiety-like behaviour and an increase in excitability of neural circuits controlling fear and anxiety (Smith et al., 1998; Brack and Lovick, 2007; Devall et al., 2009; Devall et al., 2015). The effect is mediated not by progesterone itself but via the decline in its neuroactive metabolite allopregnanolone. Interestingly, in spontaneously cycling rats the anxiogenic effects precipitated by an abrupt decline in progesterone could be prevented by offsetting the rapid fall in brain concentration by short-term treatment in the late diestrus phase with the SSRI fluoxetine (Devall et al, 2015). At low doses, fluoxetine increases brain allopregnanolone concentration without affecting 5-hydroxytryptamine systems, a property which is shared by other SSRIs (Pinna et al, 2009; Devall et al, 2009). By raising the brain concentration of allopregnanolone, administration of fluoxetine during late diestrus would offset the rapid fall in concentration of the steroid that normally occurs at this time. In this context, it is interesting that when allopregnanolone but not progesterone levels, were stabilized during the late luteal phase in women with PMDD by blocking the conversion of progesterone to allopregnanolone, luteal phase symptoms of irritability, anxiety and sadness reduced significantly (Martinez et al., 2016).

Clinically, selective serotonin reuptake inhibitors (SSRIs) are offered for the management of premenstrual syndrome. The latest Cochrane review (Majoribanks et al, 2013) rated the available evidence of only low to moderate quality but nevertheless concluded that SSRIs are effective in reducing the symptoms of PMS although adverse effects were relatively frequent. Interestingly, treatment was equally effective whether taken continuously or only during the two weeks prior to menstruation (the luteal phase). This finding raises questions regarding the mechanisms of action of SSRIs for PMS. Their effectiveness as antidepressants typically

requires a long lead in time to produce therapeutic effects via actions on 5-HT systems. Yet therapeutic effects for PMS can be obtained by short term dosing during the luteal phase (Majoribanks et al, 2013). In animal studies acute administration of SSRIs at doses subthreshold for effects on 5-HT systems has been shown to produce a rapid elevation in brain allopregnanolone (Pinna et al, 2009 Devall et al, 2015). Moreover, short-term dosing with low dose of the SSRI fluoxetine, was effective in reducing late diestrus phase anxiety (a model for PMS) (Devall et al, 2015). These findings suggest not only that the doses currently used in clinical practice, which are based on those developed for antidepressant actions, may be far higher than necessary but also that the effectiveness of SSRIs for PMS may be linked to their steroid stimulating properties, rather than uptake of serotonin.

Whilst a change in progesterone secretion during the menstrual cycle can clearly have a significant influence on brain function and behaviour in females, it may also a factor, together with actions and interactions with other gonadal hormones, that influences drug effects. There is evidence that progesterone-induced change in GABA_A receptor status during the estrous cycle may influence the effectiveness of psychoactive drugs that act on this receptor site. For example, anxiolytic effects of a benzodiazepine seen in the early stages of the estrous cycle in female rats were not evoked when the drug was given in the late diestrus phase (Soares-Rachetti et al., 2016). In a similar vein, women who suffer with premenstrual syndrome (but not asymptomatic women) showed reduced sensitivity to benzodiazepines during the luteal phase of the menstrual cycle (Bell et al., 2004; Sundstrom et al., 1997). These findings highlight the importance of taking into account female hormonal status when working towards developing a sex- specific pharmacology.

Estrogen and PTSD.

Posttraumatic Stress Disorder (PTSD) is a syndrome that can appear after exposure to a life threatening event, death, serious injury or violence. Symptoms include intrusive thoughts, nightmares, flashbacks about the traumatic event, emotional distress or physical reactivity after exposure to triggers related to the trauma, hypervigilance and heightened startle reaction (American Psychiatric Association, 2013). Patients often report experiencing feelings of reliving the traumatic event (Shearing et al., 2011; Hagenaars et al., 2009; Halligan et al., 2006). These memories trigger feelings of fear similar to those suffered during the event itself. This Impaired fear extinction or the inability to extinguish a conditioned fear response, is a hallmark of the condition (Zuj et al., 2017; Garfinkel et al., 2014; Fani et al., 2012). Gonadal steroids such as estrogen have been found to modulate the expression of fear extinction (Glover et al., 2015). Specifically, extinction deficits observed in women with PTSD correlate with low estrogen plasma levels (Glover et al., 2012) and the administration of synthetic estrogen to healthy women increased extinction recall after fear extinction (Graham and Milad, 2013). Moreover, both in healthy women and women with PTSD lower estrogen levels have been linked to impaired fear inhibition, evident in a lack of discrimination between danger and safety cues in a fear-potentiated startle paradigm (Glover et al., 2013). Estrogen levels had no effect on contingency awareness but seem to influence activity in an inhibitory neurocircuit. In female rats, estrogen enhances infralimbic control over the central amygdala during extinction recall (Maeng et al., 2017) and women with high estrogen levels exhibit increased dorsal lateral prefrontal cortex reactivity during emotional response inhibition (Amin et al., 2006). Importantly, PTSD is also characterized by diminished fear inhibition and there is converging evidence from imaging studies that patients with PTSD show hyperreactivity of the amygdala coupled with reduced top-down control by the prefrontal cortex in response to trauma- and fear-associated stimuli (Etkin

and Wager, 2007; Jovanovic and Norrholm, 2011). Recently, it has also been suggested that estrogen status may increase risk for PTSD in some women, in part through its epigenetic regulation of the HDAC4 gene, which encodes histone deacetylase 4 and is involved in long-term memory formation (Maddox et al., 2017). The apparently contradictory findings of estrogen as a protective factor versus higher PTSD prevalence rates in women, who generally have higher estrogen levels than men, underscore the necessity to consider a possible interplay between multiple hormonal systems (Zuj et al., 2016).

Role of oxytocin

The hypothalamic neuropeptide oxytocin (OXT) has often been implicated in modulating fear and stress responses (Meyer-Lindenberg et al., 2011). For example, male police officers with PTSD show lower basal salivary OXT levels than healthy trauma-exposed controls, while there is no difference in females between PTSD patients and controls (Frijling et al., 2015). By contrast, serum OXT levels in survivors of motor vehicle accidents positively predict posttraumatic coping at a 1-month follow-up in women (Nishi et al., 2015). Importantly, the intranasal administration of synthetic OXT increases OXT concentrations in the brain (Striepens et al., 2013) and may thus be used as a pharmacological means to alter central OXT signalling. Two studies showed that OXT administered intranasally before fear extinction enhanced both extinction (Eckstein et al., 2015) and the recall of extinction in healthy men (Acheson et al., 2013). This anxiolytic effect of OXT could be mediated by a down-regulation of amygdala response and a concomitant up-regulation of activity in prefrontal regions (Eckstein et al., 2015). However, there is mounting evidence that social effects of exogenous OXT are dependent upon both context and person variables (Hurlemann and Scheele, 2016). First and foremost, intranasal OXT may also increase fear conditioning when the peptide is

administered before the conditioning phase (Eckstein et al., 2016). As such, a greater risk of developing PTSD after an interpersonal trauma may be associated with an OXT-induced enhancement of fear learning. Another instance of context-dependency is the observation that OXT improves the buffering effect of social support on stress responsiveness (Heinrichs et al., 2003), but also facilitates the sensation of psychosocial stress in the absence of social support (Eckstein et al., 2014).

Sex also appears to be a key moderator of OXT effects. Sex-specific OXT effects have been found in various domains ranging from social approach/avoidance behaviour (Scheele et al., 2012; Preckel et al., 2014) and social perception (Fischer-Shofty et al., 2013) to moral decision-making (Scheele et al., 2014). Some of these sex differences could be due to hormonal fluctuations during the menstrual cycle or the use of hormonal contraception. Along these lines, a recent report showed that intranasal OXT increased a positive attractiveness bias in the perception of the romantic partner and enhanced reward-associated brain activations in men and freely cycling women, but not in women using hormonal contraception (Scheele et al., 2013; Scheele et al., 2016). Moreover, OXT increased amygdala responses to fearful faces (Domes et al., 2010) and aversive scenes (Lischke et al., 2012) in healthy women. On the flipside, OXT reduced amygdala responses to infant crying in healthy women (Riem et al., 2011), and higher doses of OXT dampened amygdala responses to fearful faces in both male and female patients with PTSD (Koch et al., 2016). Notably, the amygdala is not a homogenous structure but rather consists of various nuclei and consequently imaging with high spatial resolution may be necessary to disentangle differential OXT effects on amygdala substructures. For instance, it has been observed that OXT attenuates activation in lateral and dorsal regions of the left anterior amygdala for fearful

faces but enhances activity in a more ventral cluster for happy expressions (Gamer et al., 2010). In female mice, estrogen pre-treatment enhances anxiolytic OXT actions (McCarthy et al., 1996), indicating that anti-anxiety effects of OXT may be dependent upon interactions with steroids. Given the above mentioned evidence for facilitating effects of estradiol on fear extinction, future human studies are warranted to explore possible interactions between gonadal steroids and OXT in fear learning.

Perceived barriers to the development of sex based psychopharmacology research

Pre-clinical research in psychopharmacology is still based predominantly on male animal models of disease (Kokras and Dalla, 2014). The assumption, which is probably unfounded (Prendergast et al., 2014), of increased variability of data obtained in females compared to males has historically deterred researchers from conducting experiments on females. Moreover where researchers have used females, there are frequent contradictions in the literature because the effects of the estrous cycle have not been carefully determined to assess whether behaviours are in fact regulated in this way or not. In many cases, the behavioural tasks employed have not been validated for use with female animals which may also contribute to the variability in behavioural outcomes (Becker and Koob, 2016). Differences in the expression of symptoms between males and females extend to biological markers and these are not always acknowledged in research (Kokras and Dalla, 2017). While there is a need to include more female animals in psychopharmacology research to improve translational outcomes (Leger and Neill, 2016), these studies must be designed carefully to account for these differences. In 2014 the National Institute of Health (NIH) introduced

policies that required applicants to balance the use of male and female cells and animals in preclinical studies, unless sex-specific inclusion was unwarranted (Clayton and Collins, 2014). This move is potentially very powerful and appears to be stimulating research into both women's health issues and sex differences in disease. In the UK, the research councils have not issued the same mandate as NIH. However, there is increasing emphasis on the justification of the use of animals in research and appropriate experimental design. For example, the National Center for the Replacement, Refinement and Reduction of Animal Research (NC3Rs) experimental design hub suggests the use of an adequately powered factorial experimental design such that the effect of a drug can be tested on both males and females in the same experiment (NC3Rs). Where there is evidence of a sex difference, then it is necessary to assess the effects of gonadal steroids on that behaviour or drug treatment (Becker et al., 2005). Initially this can be achieved by establishing the effects of the estrous cycle. In rodents the estrous cycle is typically 4 days-a 2 day diestrous (rats) or metoestrus/diestrous (mice), pro-estrous (associated with luteinizing hormone surge), and estrous (sexual receptivity, ovulation) (Becker et al., 2005). Changes in cell type morphology can be assessed in vaginal smears and used to identify the estrous phase in a relatively straightforward way (McLean et al., 2012; Becker et al., 2005). As one can see, there are few barriers to using female animals in research, and a number of potential benefits for translational psychopharmacology research.

Sex differences in clinical research

In terms of clinical research, women have only been included in clinical trials relatively recently (FDA, 1993). A 2016 review of fifty-seven randomized controlled trials (RCTs) published in peer reviewed journals found that only 39% recruited women and men in equal

numbers and a meagre 20% described results by sex or examined possible sex interactions (Phillips and Hamberg, 2016). It is even more difficult to explain greater recruitment of men for illnesses that have the same rates of prevalence in both sexes or higher prevalence in women (Phillips and Hamberg, 2016; Holdcroft, 2007). Concerns about pregnancy and experimenting with women of childbearing age, while possibly justifiable (Vargesson, 2015; Balon and Riba, 2016), led to a protectionist position and the consequence was that women's health research was excluded (Mastroianni et al., 1994). Issues around ensuring the use of contraception to avoid potential fetal harm appear to complicate enrolment of women (Holdcroft, 2007). A study of contraceptive requirements for clinical research found that almost 42% of protocols did not explain why contraception was mandatory (Cain et al., 2000). There is also the possibility of interaction of between medication and hormonal contraception. In the US 28% of women of fertility age are using some type of hormonal contraception (Jones et al., 2012). Excluding women from trials may hide these effects leading to a knowledge gap about these interactions when the drug is commercialized, which could potentially lead to further harm for women.

Evidence suggests a series of social, legal, medical and perhaps institutional barriers to implementing equal recruitment in RCTs. Results of trials with biased recruitment may not apply to both sexes, but it will only be evident when the medication or intervention is released to the general public. A further concern is that researchers perceive that investigating women's physiology is more complicated because of lack of data and, specifically, the lack of replication studies (Holdcroft, 2007). This paradox can only be resolved by increasing research in the area and by encouraging scientists and institutions to prioritize sex-equal samples and the reporting of results by sex.

Sex differences in clinical practice

In psychiatric practice, sex differences are acknowledged in several areas such as domestic violence, sexual related side-effects, and perinatal mental health. Conscious sex-differential prescribing based on evidence is rare as is clinical recognition of variation of drug efficacy across sexes even when pharmacokinetic or pharmacodynamic differences are known (Legato, 2004). Recent guidelines have addressed sex differences by considering guidance for the perinatal period (NICE, 2014) but general evidence from translational research that does not refer to pregnancy or breastfeeding has yet to reach the clinician. Certain awareness in practice will be required to implement these changes. In some cases treatment protocols separated by sex may help (Mendrek and Stip, 2011). The development of a branch of psychopharmacology focused on sex differences will aid to structure the evidence and increase knowledge translation between preclinical researchers and clinical professionals until these findings are fully integrated in everyday medical practice.

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Declaration of conflict of Interest.

Dr. Bolea-Alamanac does not have any conflicts of interest to declare.

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Fig 1.A. Schematic representation of time course of change in systemic and brain progesterone concentration during the menstrual cycle. M: menses start. **B.** Time course of mean PMS score and saliva progesterone concentration in 22 symptomatic women over the

10 days prior to menstruation. ** $p < 0.01$; *** $p < 0.001$ compared to Day -10, Friedman test across all days followed by Dunn's multiple comparisons. **C.** Time course of mean PMS score and saliva progesterone concentration in 12 asymptomatic women over the last 10 days prior to menstruation. Reproduced from Lovick et al (Lovick et al., 2017) with permission.



